

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁶ : A61K 9/20, 9/28, 9/50, C08G 59/28, 63/685, 69/26, 75/24, 75/30	A1	(11) International Publication Number: WO 96/10994 (43) International Publication Date: 18 April 1996 (18.04.96)
(21) International Application Number: PCT/IB95/00834 (22) International Filing Date: 5 October 1995 (05.10.95) (30) Priority Data: 74436/94 5 October 1994 (05.10.94) AU (71) Applicant: DANBIOSYST UK LTD. [GB/GB]; Albert Einstein Centre, Highfields Science Park, Nottingham NG7 2TN (GB). (72) Inventors: SCHACHT, Etienne; Rijsselveldstraat 99, B-8840 Staden (BE). WILDING, Ian; 5 Edingale Court, Bramcote, Nottingham NG9 3LY (GB). (74) Agents: LEMAIRE, Guy et al.; Bureau Vander Haeghen - K.O.B. S.A., 108A, rue Colonel-Bourg, B-1040 Bruxelles (BE).		(81) Designated States: CA, JP, KR. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: PROCESS FOR THE PREPARATION OF COLON SPECIFIC REDUCTION SENSITIVE POLYMERS (57) Abstract Specific reduction sensitive polymers selected from the group consisting of (i) azo-containing polymers, (ii) disulfide-containing polymers, and (iii) azo- and disulfide-containing polymers, are prepared by a process comprising the steps of copolymerizing an azo- and/or disulfide-containing α,ω -difunctional reagent by polycondensation or polyaddition with a α,ω -difunctional comonomer.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

PROCESS FOR THE PREPARATION OF COLON SPECIFIC REDUCTION
SENSITIVE POLYMERS

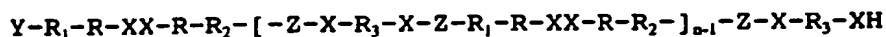
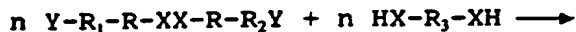
The present invention relates to a process for
5 the preparation of specific reduction sensitive polymers
selected from the group consisting of (i) azo-containing
polymers, (ii) disulfide-containing polymers, and (iii)
azo- and disulfide-containing polymers, the process com-
prising the steps of copolymerizing an azo- and/or disul-
10 fide-containing α, ω -difunctional reagent by polyconden-
sation or polyaddition with a suitable α, ω -difunctional
comonomer.

The process according to the invention has for
purpose the preparation of azo- and/or disulfide-contain-
15 ing polymers which can be completely degraded or decom-
posed into monomeric units by bacteria in the reductive
medium of the human colon intestine after a short incu-
bation period. Said polymers are useful for manufacturing
tablets and capsules intended for a site specific drug
20 delivery in the lower part of the gastrointestinal tract.

From WO91/11175, a process is already known for
preparing colon specific reduction sensitive polymers
selected from the group consisting of

- (i) azo-containing polymers;
- 25 (ii) disulfide-containing polymers, and
- (iii) azo- and disulfide-containing polymers.

Said process comprises the step of copolyme-
rizing an azo- and/or disulfide-containing α, ω -difunc-
tional comonomer according to the general reaction scheme
30 illustrated below :



35

with

-XH = -NH₂, -OH


-Y = -COOH, CO-Hal, COOAlkyl, -N=C=O,
-CH-CH(R), -SO₂Hal

5



-XX- = -N=N-, -S-S-

and

R = alkyl, aryl ()

10

R₁, R₂ = alkyl, aryl, alkylaryl groups optionally substituted

R₃ = alkylidene, arylidene, alkylarylidene
optionally substituted
polyether, polyester

15

hal = halogen radical, e.g. Cl, Br

with



and

20 Z = C=O, CH₂-CH-OH, SO₂

and whereby

X and Y are interchangeable in the above formulas.

25 Said process allows the preparation of a multitude of azo- and/or disulfide-containing polymers through a variation in the α,ω -difunctional reagents. The final physicochemical and physical properties (hydrophilicity, permeability, thermal properties, rheological properties)
30 can be widely varied.

The molecular weight of the resulting polymers is advantageously determined by gel permeation chromatography. It can be altered by adjusting the degree and type monomers used in the reaction mixture.

35 The molecular weight of the polymers prepared by the process of the invention is adjusted by modifying

the molar ratios of the functional groups reacting with one another. For high molecular weight polymers equivalent amounts of reactive functional groups should be used.

5 It is well known to those skilled in the art that molecular weights for polymers must be above about 1000 to avoid adsorption in the gastrointestinal tract and that polymers for use in the gastrointestinal tract can have average molecular weights up to 10,000,000. (See
10 U.S. Patent No. 4,298,595, Col. 25, lines 14-33).

The polymers are especially appropriated for preparing specific drug delivery systems. The latter can be matrix type systems or reservoir type systems. In the former case the azo- and/or disulfide-containing polymer
15 is a major part of the drug containing compartment. In the latter part the azo- and/or disulfide-containing polymer is used to encapsulate a drug loaded core.

Owing to the property that azo- and/or disulfide polymers are stable in the fluids of the mouth,
20 the stomach and the upper intestine the polymers according to the invention can pass the mouth, stomach and upper intestine without being destructed. In the lower part of the gastrointestinal tract, these reduction sensitive polymers are too slowly cleaved and fragmented by
25 the reductive medium, all or not enzym mediated, so that enclosed active agent is not completely released at the site of degradation.

Several segmented disulfide-containing polyamides and hydrophilic groups suitable for reductive
30 cleavage were prepared by polycondensation of 3,3'-dithiobissuccinimidylpropionate and α,ω -amino-terminated poly(tetramethyleneoxide) or tetraethyleneglycol diamine (JEFFAMINE EDR-192). Polymer films were incubated in a
15 ml sample taken from a SHIME reactor, t.m. Simultating
35 Human Intestinal Microbial Ecosystem, which is simulating

the microbial contents of human colon.

Also, segmented polyurethanes comprising disulfide and hydrophilic groups intended for the preparation of capsules which specific dry delivery were synthesized by reaction of 4,4'-diphenylmethane diisocyanate (MDI), an α,ω -hydroxy-terminated prepolymer and a disulfide-containing chain extender. As hydroxy-terminated prepolymers were used poly(tetramethyleneoxide), poly(propyleneoxide), polycaprolactone, poly(ethyleneoxide), poly(ethyleneoxide/polypropyleneoxide).

In a same manner, segmented polyurethanes comprising azo-aromatic and hydrophilic groups were synthesized by reaction of m-xylylene diisocyanate with a mixture of m,m'-dihydroxyazobenzene, poly(ethylene glycol) and 1,2-propanediol. A hydrophilic drug (FOY-305) was enclosed in a capsule made of these polymers or coated with these polymers. The capsules or coated pellets were incubated in a SHIME-reactor reproducing a culture of human intestinal flora. Samples of reduction sensitive polymers are taken over time at measured intervals. The degradation in molecular weight is observed by performing gel permeation chromatography on the samples. Although the drug was released from these pellets, the molecular weight did not decrease substantially. Hence there was no degradation of the main chain azo groups to amine groups.

A series of azo-containing polyamides were prepared by polycondensation of an azo-containing diacid chloride with an oligomeric α,ω -diamine. The polymers were incubated in an in-vitro reductive medium. A plurality of tests have indicated that numerous azo-containing polymers become sticky and colourless during incubation in a SHIME-reactor simulating a culture of human intestinal flora. Changes in structure were monitored by ^{13}C , Raman, UV and GPC analysis. The observed discolouring of these polymers is not necessarily due to degradation, but

can be due to partial reduction of the azo to the hydrazo form.

The thermodynamic conditions for the reductive cleavage of the azo polymers were determined by voltametric measurements.

It was shown that for the hydrophilic azo-containing polyamide, incubation in the reductive medium led to degradation of the azo bond with formation of amines.

The molecular weight of the azo-polymers does not decrease substantively. The partial reduction of azo- to hydrazo-group occurs slowly and there is no degradation of the main claim azo groups to amine groups.

Duration incubation, the polymers become sticky and colourless. During reoxidation, the original colours reappears and the molecular weight raises to the initial value.

These experiments indicate that for this type of polyamide no degradation of the azo-bond to amino-groups occurs. Changes in colour and mechanical properties are due to a conversion of the azo- to the hydrazo-groups. These finding are in good agreement with those of Kimura et al. in EP-A-0 398 472. They have shown in an article published in Polymer 33(1992)5294-5299 that by incubation of azo-containing polyurethanes in a culture of human intestinal flora, no molecular weight decrease occurred. As from these results, they concluded that there was no degradation of azo groups to amines, only to hydrazo groups.

The present invention aims to improve the thermodynamic conditions for the reductive cleavage of azo or disulfide containing polymers by incubation of said polymers in a similar reductive medium.

To achieve this improvement, the present invention proposes a process of the type described in the preamble of claim 1. This process is characterized in that

HX-R₃-XH is a hydrophilic comonomer
with

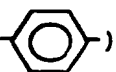
-XH = -NH₂, -OH

5 -Y = -COOH, CO-Hal, COOAlkyl, -CH-CH(R), -SO₂Hal

$$\begin{array}{c} \backslash / \\ \text{O} \end{array}$$

-XX- = -N=N-, -S-S-

and

10 R = alkyl, aryl ()

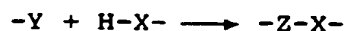
R₁, R₂ = alkyl, aryl, alkylaryl groups optionally substituted by methoxy-groups

R₃ = alkylidene, arylidene, alkylarylidene all or not substituted

15 polyether, polyester

hal = halogen radical, e.g. Cl, Br

with



and

20 Z = C=O, CH₂-CH-OH, SO₂

and whereby

X and Y are interchangeable in the above formulas.

25 According to a particularity of the invention, the process comprises the copolymerization of dithio bis-(succinimidyl propionate) with a hydrophilic α,ω -difunctional comonomer.

The reduction sensitive polymers are prepared according to the methods described hereunder.

30 Description of the starting materials

Jeffamine ED-600 (Mn=600) (7) is a commercial product purchased from Texaco Chemical Company (Austin, USA). The polymer is dried by azeotropic removal of water from toluene solution.

35 The amine content is determined by titration :
Jeffamine ED-600 : 2.96 meq/g.

Azobenzene-4,4'-diacid chloride (5) was prepared from the corresponding azobenzene-4,4'-dicarboxylic acid (4) as described in the literature [23]. All other reagents were obtained from Janssen Chimica (Beerse, Belgium).

The chloroform was washed with sulfuric acid and water and subsequently dried over calciumhydride.

Triethylamine was purified by reacting with tosylchloride and ninhydrin, respectively.

It was finally dried over calciumhydride.

Methods

IR spectra were recorded on a Beckmann IR 4230 apparatus. UV spectra were measured on a Kontron Uvikon 810 double beam spectrophotometer.

¹H-NMR spectra were recorded using a 360 MHz Bruker WH-360 apparatus.

¹³C-NMR spectra were obtained at 100 MHz on a Varian Unity-400 spectrometer using a 5 mm broadband probe.

The molecular weight of the polymers was determined by gel permeation chromatography. A PL gel mixed D (5 μm) column was used with chloroform as eluent. For calibration, polystyrene standards were used.

FT-Raman spectra were recorded using a Bruker IFS 66FT IR spectrometer equipped with a FRA 106 FT Raman module.

Voltametric measurements were carried out on a EG&G Princeton Applied Research Potentiostat/Galvanostat Model 273 using a Pd electrode as working electrode and a saturated calomel electrode (SCE) as reference electrode.

Viscosity was measured using a Ubbelohde viscosimeter (Haake).

The preparation of a polyamide starting from Jeffamine ED-600 and azobenzene-4,4'-diacid chloride

is achieved according to a method described by M. BALASUBRAMANIAN et al., in Makromol. Chem. 180 (1979) 2517-2519. The 10 g (14.80 mmol) of Jeffamine ED-600, dissolved in 100 ml dry, alcohol free chloroform, was placed in a three necked flask and stirred under nitrogen atmosphere. To this solution 7 ml (50 mmol) triethylamine was added. The mixture was stirred for 15 min at -10°C. Then 4.4 g (13.03 mmol) azobenzene-4,4'-diacid chloride, dissolved in 100 ml, dry ethanol free chloroform, was added at once at the reaction mixture. This mixture was stirred for 15 minutes at -10°C and then allowed to reach room temperature. Stirring was continued for 24 hours. The reaction mixture was extracted with 0.1 M HCl (3 x 150 ml), water, 0.1 M NaOH (3 x 150 ml). The chloroform layer was dried over MgSO₄, filtered and evaporated to dryness. The resulting polymer was obtained as an orange coloured waxy solid.

¹H NMR spectroscopy (CDCl₃; 360 MHz) : δ = 1,1 ppm (d, CH₃, γ of PPG ether); δ = 1,35 ppm (d, CH₃, β of PPG ether); δ = 3,65 ppm (m, CH₂, α of PEG ether); δ = 3,8 ppm (m, CH β of PPG ether); δ = 4,4 ppm (broadened, N-H amide); δ = 7,95 ppm (m, aromatic protons).

IR-spectroscopy (film on KBr discs) : ν = 3300 cm⁻¹ N-H stretching vibration, ν = 1650 cm⁻¹ C=O stretching vibration, ν = 1540 cm⁻¹ N-H bending vibration, ν = 1600 cm⁻¹ C-H aromatic stretching vibration, ν = 1100 cm⁻¹ C-O stretching vibration.

Casting of films

A concentrated solution (ca. 30%) of the azo polyamide in chloroform was casted on a siliconized glass plate. After evaporation of the solvent in a hot air ventilated oven, the film was removed from the glass plate.

Preparation of in-vitro reductive medium

A solution of sodium phosphate buffer (0.25 M, pH 6.5) was boiled and further cooled under an atmosphere

of Argon. While stirring, sodium sulphide and cystein are added to give concentrations of 50 mM for each.

The redox potential of this medium, determined with a Pt redox-electrode, was -430 mV (± 10 mV) and remained constant for at least 4 days. The reductivity of this medium was assayed by incubation of sulfalazine. The formed 5-ASA was determined qualitatively and quantitatively by HPLC analysis.

Incubation of films in chemical reductive medium

An azo polyamide ca. 0,1 g (thickness ± 100 μ m) was incubated in a solution of 30 ml reductive medium for periods from 3 hours varying to 2 days. At regular time intervals, films were removed from the medium, washed with water, dried in vacuo and analysed by GPC. FT-Raman and ^{13}C -NMR measurements.

Incubation of films in simulating human intestinal medium (SHIME)

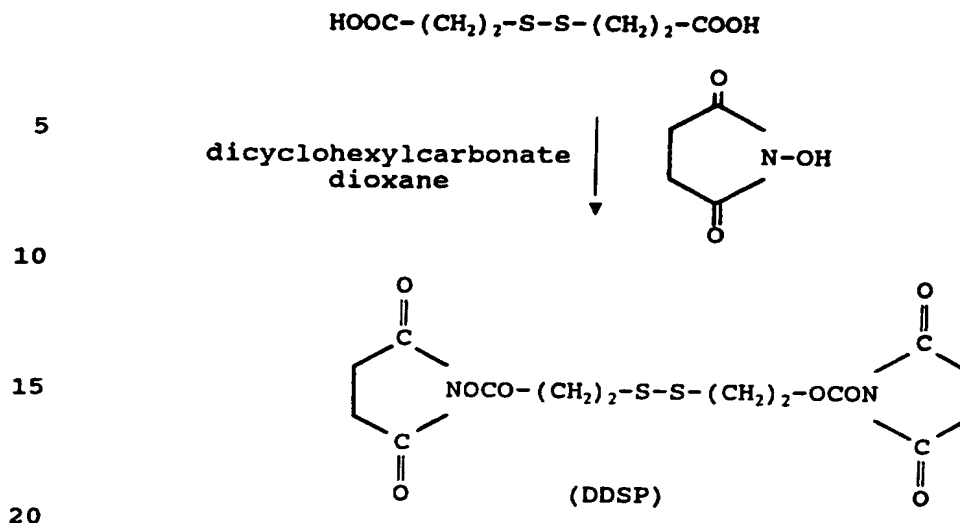
An azo polyamide ca. 0.05 g (thickness ± 100 μ m) was incubated in 15 ml batch medium of a SHIME-reactor for periods from 12 hours varying to 3 days. The SHIME-reactor consists of 5 vessels which represent the ileum (2 vessels) and the colon (3 vessels). The redox potential -250 mV (± 50 mV) is daily measured with a Pt redox-electrode. The incubation flasks were kept at 37°C. At regular time intervals, films were removed from the medium, washed with water, dried in vacuo and analysed by GPC, Raman and ^{13}C -NMR measurements.

Results and Discussion

I) Synthesis of co-polyamides containing disulfide

The disulfide-containing polymers were prepared by polycondensation of 3,3'-dithiobissuccinimidyl propionate and α,ω -amino terminated prepolymer.

3,3'-dithiobissuccinimidyl propionate (DDSP) was synthesized according to the following reaction schema :



To a solution of 5 gm (23.778 mmol) 3,3'-di-thiobissuccinimidyl propionate (DDSP) in 125 ml dry dioxane in 250 ml two neck flask under nitrogen atmosphere was added a solution of 6.25 gm (54.3 mmol) N-hydroxysuccinimide (NHS). The reaction mixture was cooled to 0°C in an ice-acetone bath followed by addition of 11.25 gm (54.52 mmol) dicyclohexylcarbonate (DCC). The reaction mixture was stirred for 1 hour at 0°C and the stirring was continued overnight at room temperature. The precipitated dicyclohexylurea (DCU) was filtered off, washed with dry dioxane, the filtrate was concentrated to one-third and was left overnight in refrigerator.

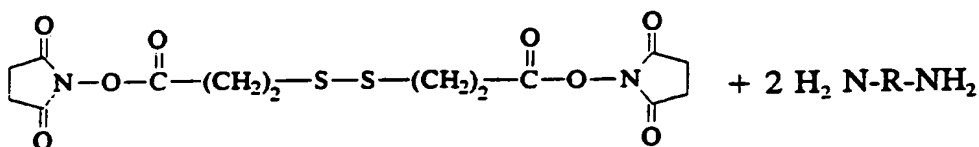
The precipitated traces of DCU was filtered off and the filtrate was evaporated on rotary evaporator. The white crude product was recrystallized by refluxing it in a mixture (150 ml) of dry acetone and diethyl ether (1:1) for 1-2 hrs. The DCU impurities was found to be more soluble in the refluxing mixture and the pure DDSP was less soluble, the insoluble product was filtered and dried in vacuum. The last step was repeated 2-3 times

till no DCU was evidenced in the NMR of the product.

B) Copolymerization of DDSP with Jeffamine EDR-192 and PTHF-diamine 750

B-1) 1:1 PTHF-750 : EDR-192

5 2.



10

{
50% (PTHF-750)
50% (JEFFAMINE EDR 192)

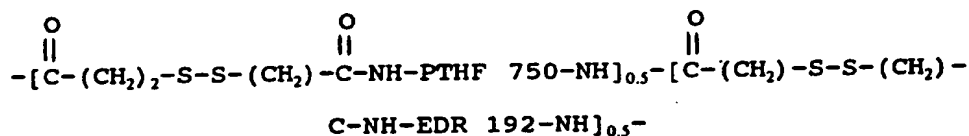
15

20

CHCl₃

TEA

25



with EDR-192 = H₂N(CH₂CH₂O)₃CH₂CH₂NH₂

PTHF 750 = H₂N-(CH₂)₄-O-[CH₂CH₂CH₂CH₂O-]_n-(CH₂)₄NH₂

30

Procedure

To a cooled solution (0°C) of 2.51 gm (24.75 meq) EDR-192 in 15 ml dry chloroform, 10.55 gm (24.75 meq) PTHF-750 in 15 ml dry CHCl₃, and 13.69 ml triethylamine was added a solution of DDSP (49.5 meq) in 65 ml CHCl₃. The reaction mixture was stirred at 0°C for 1 hour and the stirring was continued for 36 hrs at room temperature.

The chloroform layer was extracted with HCl 0.1 M (3x), H₂O (3x), NaOH 0.1 M (3x) and finally with H₂O (3x). The CHCl₃ layer was dried over MgSO₄ overnight and

- 5 1) Good film forming
- 2) GPC (NMP) : Weight average molecular weight : 21791
 Dispersity : 2.3
- 3) Colonic degradation : see Table 1.

Polymer films were incubated in a 15 ml sample taken from a SHIME reactor. The SHIME reactor (Simulating Human Intestinal Microbial Ecosystem) is simulating the microbial contents of human colon. The flasks were degassed by flushing it with nitrogen and then was incubated at 37°C. At regular times samples were taken and analyzed by GPC (eluent : NMP). Undissolved polymer samples were washed, dried under vacuum and the analyzed by GPC. The soluble polymers were removed by freeze-drying the medium and the residue was dissolved in NMP, filtered to avoid inorganic salts and the analyzed by GPC.

TABLE 1
Colonic degradation of copolymer (1:1):(efr. B-1)

Incubation time (hrs)	Insoluble part			Soluble part		
	Mw	Mn	Polydisp.	Mw	Mn	Polydisp.
0.0	21884	6104	3.58			
0.5	15162	2594	5.84			
1.0	14492	2621	5.52			
2.0	13046	2318	5.62	3557	789	4.5
4.0	11022	2090	5.27			
6.0	12398	2047	6.057	2893	731	3.958
26.5	14101	2550	5.53	2835	718	3.948
47.0	1341	2220	6.188	1921	717	2.679

Comment : Slow degradation.

III) Mechanical tests

Tensile testing

The tensile strength of the polymer samples was measured using a Hounsfield type H10KM tensile machine. The samples was tested at 25°C at a crosshead speed (tensile rate) of 20 mm/min using 100 N cell.

Film preparation

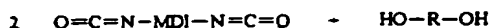
Polymer solutions containing 10% w/w of the polymer in chloroform were cast on a Téflon plate. The plate was covered with a watch glass to slow down solvent evaporation, solidified by atmospheric air drying. The films were carefully removed from the plate and dried to constant weight in vacuum at 30°C. The thickness of the film was measured using micrometer Forster-isometer S 2.320 (Belgium). The film was cutted for tensile strength measurements using hand press cutting knife from Berg & Schmid model HK500.

1.3 Disulfide-containing polyurethanes

The disulfide-containing polyurethanes are pre-

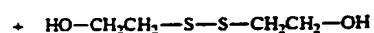
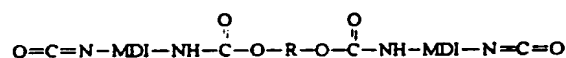
pared by polycondensation of 4,4'-diphenylmethane diisocyanate (MDI), an α,ω -hydroxy-terminated prepolymer and a disulfide-containing chain extender, according to the following reaction scheme :

5

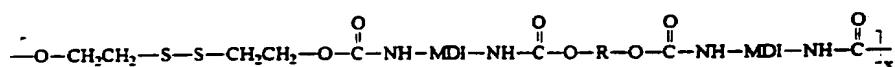


DMAc

10



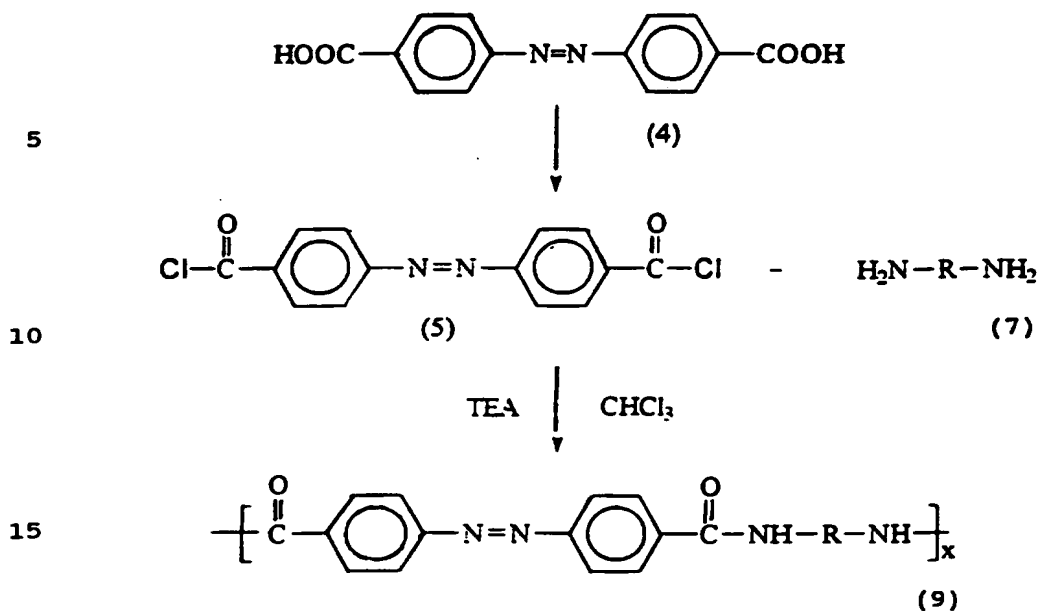
15



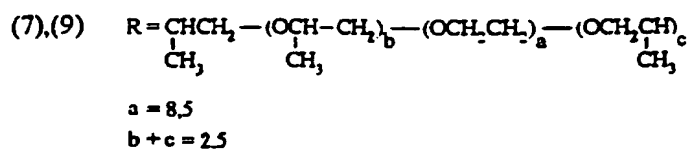
As hydroxy-terminated prepolymers were used :
poly(tetramethyleneoxide), poly(propyleneoxide), poly(ca-
prolactone), poly(ethyleneoxide), poly(ethyleneoxide/pro-
pyleneoxide).

Synthesis of azo-containing polyamides

The azo-containing polymers were synthesized by
polycondensation of azobenzene-4,4'-diacid chloride and
oligomeric α,ω -diamines in chloroform as solvent and tri-
ethylamine as proton acceptor. The azobenzene-4,4'-diacid
chloride was prepared from the corresponding azobenzene-
4,4'-dicarboxylic acid by reaction with thionylchloride
in toluene as solvent. The oligomeric diamines selected
was hydrophylic (Jeffamine ED-600) (7) which resulted in
water soluble (9) polymer :



20



25

30 Characterisation of the polymers was done by ¹H-NMR and IR spectroscopy. Table 1 shows the molecular weight of both azo polymers as determined by GPC using polystyrene as standards.

TABLE 1
Molecular weight of the azo-containing polyamides

	Polymer	Diamine	Molecular weight
5	Hydrophylic azo polymer	Jeffamine ED-600	Mw = 16500 Mn = 7900

Reduction of azo-containing polyamides in chemical reductive medium

10 The degradation of the azo polymers was investigated in an in-vitro reductive medium. During incubation, the films become sticky and colourless. Upon drying of the incubated polymer at 50°C in air, the samples showed a rapid colour recovery from colourless to orange.

15 Table 3 shows molecular weight as determined by GPC using polystyrene as standards and the results of viscosity measurements for said hydrophylic azo polymer (9) before and after incubation in a chemical reductive medium.

20

TABLE 3
Changes in molecular weight of after incubation in the chemical in-vitro reductive medium

25	Incubation time (h)	Mw	Mn
	0	16380	7280
	6	4410	2120
	24	4230	1880
	30	3790	1730
30	48	2860	1215
	72	2060	940

GPC analysis of samples taken at different incubation times (table 3) show a remarkable decrease in

molecular weight. After 3 hours incubation, the molecular weight is apparently reduced of a factor 10 faible original value and remains so for the further 48 hours of incubation (see table 3). However, during reoxidation, the original colour appears and the molecular weight as determined by GPC raises to the initial value. The films dissolved completely in the reductive medium after 6 hours of incubation.

5 Spectroscopic evidence for the formation of the hydrazo bond during reduction of the azo bond

10 Evidence for the formation of hydrazo groups during reduction of the hydrophobic azo polymer (8) was gained by ^{13}C -NMR analysis.

15 Table 4 gives different chemical shifts for the oxidized and reduced form of the hydrophobic azo polymer. The aromatic carbon resonances were assigned based on additivity rules [25] using a para X-Ph-Y structure with Y = -N=N-Ph and X = -CON(CH₃)₂ for the oxidized polymer and with Y = -NH-NH₂ and X = -CON(CH₃)₂ for the reduced one. The calculated values are in good agreement with those determined experimentally for the oxidized and the reduced form.

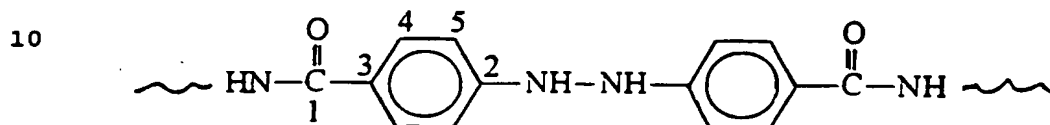
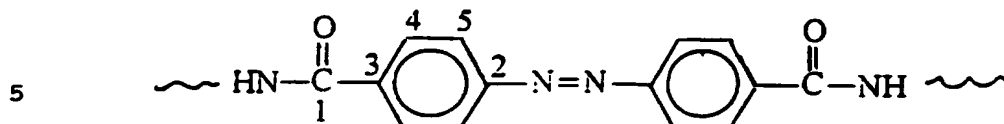
20 In conclusion it can be stated that both polymer forms, having a few characteristic carbon resonance lines (e.g. C3 and C5), can easily be discriminated from each other by ^{13}C -NMR.

25 The ^{13}C -NMR spectra of the reoxidized polymers, after incubation in the chemical reductive medium and in the SHIME reactor, are clearly mixtures of the reduced and oxidized forms. The polymer which was incubated in the chemical reductive medium, reoxidized for three days in air atmosphere, still contains mainly (about 90%) of the reduced form. The polymer which was incubated in the SHIME-reactor, reoxidized for about two weeks in air atmosphere, contains mainly (about 55%) of the oxidized

30

35

form.

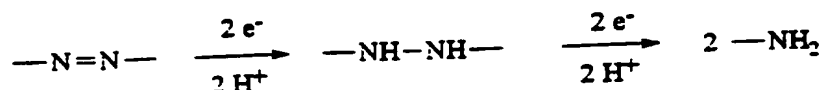


15 FT-Raman spectroscopy is an attractive tool to study the reduction of the azo groups. The aromatic ring vibrations in both spectra appear at 1613 cm⁻¹ and 1607 cm⁻¹. The typical Raman shift at 1460 cm⁻¹ can be assigned to the N=N functional group and is present in the oxidized form. This Raman signal can be used to differentiate between the oxidized and reduced compounds. FT-Raman can thus be applied as a complementary non-destructive technique for this purpose. For the reduced polymer the N=N stretching band at 1460 cm⁻¹ is missing and replaced by a broad band at 1298 cm⁻¹. This band is due to the symmetrical NH-NH stretching. The reoxidized polymers contain a shift at 1460 cm⁻¹, characteristic for the N=N group. However, the higher relative intensity of the Raman line at 1460 cm⁻¹ of the in SHIME- reactor incubated polymer can be correlated with a higher amount of the oxidized polymeric form. These results are totally in agreement with the ¹³C-NMR data.

Voltametric experiments

35 Literature data [28-29] show that the electrochemical reduction of an azo bond proceeds via a two-

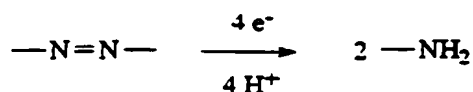
electron step mechanism towards the hydrazo group. Reduction surpassing the hydrazo-derivatives generally requires more energy than the azo-hydrazo reaction. If the difference in energy is great enough, the reduction may
5 be represented by two waves (figure 5).



10

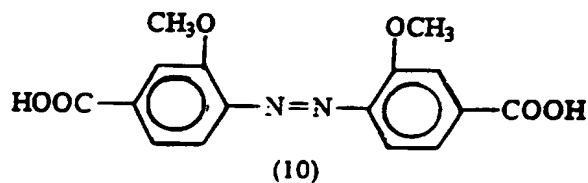
In some cases, however, azo compounds are known to be reduced to the amines, by a four-electron step reaction (figure 6).

15



Voltametric measurements have been carried out
20 in water, in which tetrabutylammoniumbromide was used as electrolyte. By using water as a solvent, only the water soluble, hydrophylic polymer (9), could be investigated. From table 5 we can notice that the redox potential of (9) is -230 mV vs. SCE (+ 6 mV vs. SHE). This explains
25 why the polymer is reduced in the chemical reductive medium.

Groups such as hydroxy, amino, methoxy, which increase the electron density on the azo linkage, show a shift towards higher (less negative) redox potentials and
30 proceed by a four-electron step reduction towards amines [30,31]. Voltametric measurement of the water soluble azobenzene-4,4'-dicarboxylic acid (4) showed a lower half wave potential than the methoxy substituted analogue (10).



There is a difference of 140 mV in redox potential between both products. This implies that the reduction of the methoxy substituted diacid is more favourable.

10

TABLE 4
Half wave ($E_{1/2}$) potentials

15

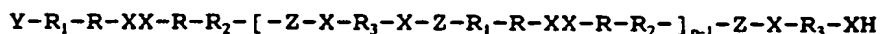
Product	$E_{1/2}$ (mV) vs. SCE	$E_{1/2}$ (mV) vs. SHE
9	-230	+6
<p>20</p>	+130	+366
<p>25</p>	+270	+506

When the hydrophylic azo polymer is incubated in a similar reductive medium, degradation proceeds with reduction of azo groups to amines. The solubility of the azo polymers in the reductive medium is the determining factor. Voltametric measurements have shown that all water-soluble azo compounds have a redox potential, more

30

positive than the reductive medium. These results indicate that the thermodynamic conditions for the reductive cleavage of azo bonds are fulfilled.


(i) azo-containing polymers;
(ii) disulfide-containing polymers, and
(iii) azo- and disulfide-containing polymers,
comprising the step of copolymerizing an azo- and/or
disulfide-containing α,ω -difunctional reagent by polycon-
densation or polyaddition with a suitable α,ω -difunctio-
nal comonomer according to the general reaction scheme
illustrated below :



20

$$-XH = -NH_2, -OH$$

-XX- = -N=N-, -S-S-

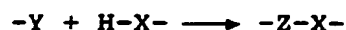
R = alkyl, aryl ()

30

R₃ = alkylidene, arylidene, alkylarylidene all or not substituted

hal = halogen radical, e.g. Cl, Br

with



and

Z = C=O, CH₂-CH-OH, SO₂

5 and whereby

X and Y are interchangeable in the above formulas.

2.- The process according to claim 1, characterized in that it comprises the copolymerization of dithio bis (succinimidyl propionate) with a hydrophylic
10 α,ω -difunctional comonomer.

3.- The process according to claim 1 or 2, characterized in that it comprises the copolymerization of dithio bis (succinimidyl propionate) with a hydrophylic α,ω -diamine functional comonomer.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 95/00834

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/20 A61K9/28 A61K9/50 C08G59/28 C08G63/685
C08G69/26 C08G75/24 C08G75/30

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO.A,91 11175 (DANBIOSYST UK LTD) 8 August 1991 cited in the application see the whole document -----	1-3

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

22 January 1996

Date of mailing of the international search report

19. 02. 96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Boeker, R

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 95/00834

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9111175	08-08-91	BE-A- 1003677	19-05-92
		DE-D- 69100535	25-11-93
		DE-T- 69100535	11-05-94
		EP-A- 0513035	19-11-92
		US-A- 5407682	18-04-95
